The Answer To a 100-Year Old Puzzle: The Structural Basis for Specificity in Human ABO(H) Blood Group Biosynthesis

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Scientists working at beamlines X4A, X8C, and X12C have determined to a high resolution the structures of the enzymes that synthesize the human A and B blood group antigens. Although a mismatched blood transfusion could be fatal, the researchers found that the differences between the two enzymes are surprisingly small.

More than 100 years ago, Austrian pathologist Karl Landsteiner first investigated why some blood transfusions succeeded in reviving patients who had suffered severe blood loss while other transfusions killed. In 1901, he discovered the A, B, and O blood groups, for which he was rewarded with the Nobel Prize in physiology or medicine in 1930. Given the potentially fatal consequences of mismatched blood types, scientists presumed for more than half a century that the A and B blood groups must be significantly different from each other. But in 1956, the A, B, and O blood group antigens (antigens are molecules recognized by the immune system) were all found to be carbohydrate structures present on cell-surface glycoproteins (protein with attached sugars) and glycolipids (lipids with attached sugars).

The A and the B antigens were found to be modified from the carbohydrate corresponding to the O blood group by the addition of different monosaccharides (simple sugars). The A antigen was terminated by the sugar N-acetylgalactosmine (GalNAc) while the B antigen was terminated by galactose. In essence, these two blood groups, which were so immunologically distinct, differed only in the replacement of an acetamido group ($-NHCOCH_3$) by a hydroxyl group (-OH), as shown in **Figure 1**.

The inherited nature of the A, B, and O blood groups meant that the A and B antigens had to be produced by genetically encoded enzymes. In 1959, scientists postulated the existence of specific glycosyltransferases, enzymes that catalyze the transfer of sugar units between molecules. Their existence was later demonstrated in 1966, 1967, and 1968. In general, individuals with blood group A carried the gene for glycosyltransferase A (GTA), those with blood group B carried the gene for glycosyltransferase B (GTB), those with blood type AB carried both genes, and those with blood type O carried neither or an inactive gene.

In 1990, scientists cloned the genes corresponding to the glycosyltransferase enzymes that produce the A and B antigens and found that these enzymes are membrane-bound proteins of about 330 amino acids (elementary units of proteins) in length that differed from each other by only four amino acids. Both enzymes would recognize the disaccharide H antigen acceptor (corresponding to blood type O), so scientists thought that these four amino acid differences must serve to recognize the two different monosaccharide donors: uridine diphosphate-GalNAc for GTA, and uridine diphosphate-galactose for GTB.

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Later, kinetic characterization of GTA and GTB mutants revealed which of the four amino acid residues contributed most in distinguishing between the two donor molecules.

Using the very intense x-rays at beamlines X4A, X8C, and X12C, the structures of the GTA and GTB enzymes have been solved, in order to further characterize how they work.

Of the four amino acids that differ between GTA and GTB, only two (leucine/methionine 266 and glycine/alanine 268) are located in the active site pocket of both enzymes and well-positioned to contact the donor sugars, as shown in **Figure 2**. Remarkably, these small differences are enough to make the two enzymes use different mechanisms to recognize their respective donors.

The active site pocket of GTB contains the larger residues methionine 266 and alanine 268, used to exclude the GalNAc donor, and the active site in GTA contains the smaller residues leucine 266 and glycine 268, which can easily accommodate the galactose as well as GalNAc. GTA can easily recognize its donor because the smaller leucine 266 exposes the nearby residue histidine 233, which can form a strong hydrogen bond with the acetamido group of GalNAc, but cannot contact galactose. Thus, while GTB functions by excluding the incorrect donor, GTA functions by recognizing the correct donor.

Because such a small difference in enzyme and antigen structures has such large physiological consequences, GTA and GTB are paradigms for specificity in biosynthetic and immune recognition, paving the way for the design of enzymes with different specificities.
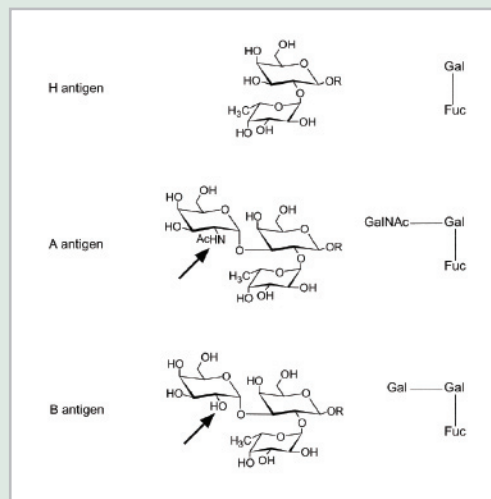
Figure 1. The antigens corresponding to the A, B, and O blood groups. The A and B antigens differ from the H antigen by the addition of different monosaccharides to the H antigen (corresponding to blood group O). Remarkably, the A and B antigens differ only by the replacement of an acetamido group (-NHCOCH$_3$) in A with a hydroxyl group (-OH) in B.
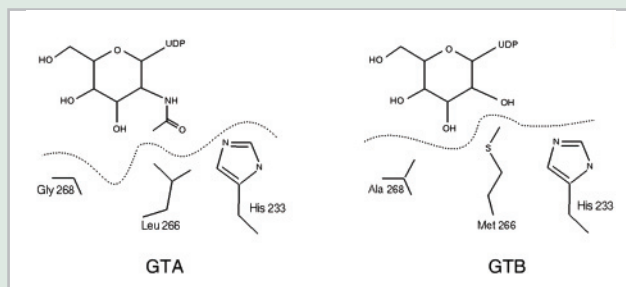
Figure 2. Leucine/methionine 266 and glycine/alanine 268 are the only amino acid residues that differ between the active sites of glycosyltransferase A (GTA) and glycosyltransferase B (GTB). While GTB excludes the A monosaccharide donor based on size, GTA selects the correct donor by forming a hydrogen bond with histidine 233.